Safety, tolerability, and immunogenicity of the Ebola Sudan chimpanzee adenovirus vector vaccine (cAd3-EBO S) in healthy Ugandan adults: a phase 1, open-label, dose-escalation clinical trial

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Summary

Background Sudan Ebola virus can cause severe viral disease, with an average case fatality rate of 54%. A recent outbreak of Sudan Ebola virus in Uganda caused 55 deaths among 164 confirmed cases in the second half of 2022. Although vaccines and therapeutics specific for Zaire Ebola virus have been approved for use during outbreak situations, Sudan Ebola virus is an antigenically distinct virus with no approved vaccines available.

Methods In this phase 1, open-label, dose-escalation trial we evaluated the safety, tolerability, and immunogenicity of a monovalent chimpanzee adenovirus 3 vaccine against Sudan Ebola virus (cAd3-EBO S) at Makerere University Walter Reed Project in Kampala, Uganda. Study participants were recruited from the Kampala metropolitan area using International Review Board-approved written and electronic media explaining the trial intervention. Healthy adults without previous receipt of Ebola, Marburg, or cAd3 vectored-vaccines were enrolled to receive cAd3-EBO S at either 1×10^{10} or 1×10^{11} particle units (PU) in a single intramuscular vaccination and were followed up for 48 weeks. Primary safety and tolerability endpoints were assessed in all vaccine recipients by reactogenicity for the first 7 days, adverse events for the first 28 days, and serious adverse events throughout the study. Secondary immunogenicity endpoints included evaluation of binding antibody and T-cell responses against the Sudan Ebola virus glycoprotein, and neutralising antibody responses against the cAd3 vector at 4 weeks after vaccination. This study is registered with ClinicalTrials.gov, NCT04041570, and is completed.

Findings 40 healthy adults were enrolled between July 22 and Oct 1, 2019, with 20 receiving 1×10^{10} PU and 20 receiving 1×10^{11} PU of cAd3-EBO S. 38 (95%) participants completed all follow-up visits. The cAd3-EBO S vaccine was well tolerated with no severe adverse events. The most common reactogenicity symptoms were pain or tenderness at the injection site (34 [85%] of 40), fatigue (29 [73%] of 40), and headache (26 [65%] of 40), and were mild to moderate in severity. Positive responses for glycoprotein-specific binding antibodies were induced by 2 weeks in 31 (78%) participants, increased to 34 (85%) participants by 4 weeks, and persisted to 48 weeks in 31 (82%) participants. Most participants developed glycoprotein-specific T-cell responses (20 [59%, 95% CI 41–75] of 34; six participants were removed from the T cell analysis after failing quality control parameters) by 4 weeks after vaccination, and neutralising titres against the cAd3 vector were also increased from baseline (90% inhibitory concentration of 47, 95% CI 30–73) to 4 weeks after vaccination (196, 125–308).

Interpretation The cAd3-EBO S vaccine was safe at both doses, rapidly inducing immune responses in most participants after a single injection. The rapid onset and durability of the vaccine-induced antibodies make this vaccine a strong candidate for emergency deployment in Sudan Ebola virus outbreaks.

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Introduction

Over 40 known human outbreaks of *Filoviridae* viruses have occurred, with increasing regularity since their discovery in 1976.[1] Two genera of viruses within the *Filoviridae* family are known to infect humans: *Ebolavirus* and *Marburgvirus*.[2] Sudan Ebola virus and Zaire Ebola virus are the two species of the *Ebolavirus* genus responsible for the majority of outbreaks of Ebola virus

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See Online for appendix

Research in context

Evidence before this study

Sudan Ebola virus, along with Zaire Ebola virus, are the two species of the *Ebolavirus* genus of the *Filoviridae* family responsible for the majority of human outbreaks of Ebola virus disease. There have been eight recorded outbreaks of Sudan Ebola virus, including a recent outbreak in Uganda that was active between September, 2022, and January, 2023. Despite the availability of approved vaccines and therapeutics for Zaire ebolavirus (Zaire Ebola virus), there are none approved for use against Sudan Ebola virus. Therefore, there is a pressing need to evaluate vaccine candidates specific to Sudan Ebola virus. We searched PubMed from Jan 1, 1990, through Nov 4, 2022, using search terms "Sudan", "Ebola", "SUDV", "vaccine", and "clinical trials". The search yielded reports from phase 1 and 2 clinical trials of several vaccine platforms including DNA, human Ad5-vectored or Ad26-vectored vaccines, and chimpanzee (c)Ad3-vectored vaccines. Although trials for the DNA vaccines and Ad5 vector vaccines had favourable safety results, these platforms were suboptimal due to the low immunogenicity of the DNA platform (necessitating multiple doses) and high population immunity against the Ad5 vector (resulting in significantly decreased glycoprotein-specific responses in individuals with high pre-existing antibody titres to Ad5). An MVA-BN-Filo boost (containing Zaire, Sudan, and Marburg antigens) following a Zaire-specific Ad26.ZEBOV prime has been shown to induce responses against Sudan Ebola virus following regimen completion, although this regimen is not approved for use against Sudan Ebola virus. A trivalent pan-filo Ad26.Filo prime and MVA-BN-Filo boost completed phase 1 evaluation in 2018, showing safety and immune responses against Zaire, Sudan, and Marburg antigens following regimen completion. However, a 56-day prime-boost interval makes these regimens less desirable during an outbreak response. Another vaccine with published results is a bivalent cAd3-EBO vaccine expressing both Zaire and Sudan glycoproteins, the Sudan component of which is evaluated as a monovalent vaccine in this report. The cAd3 vaccine platform has a favourable safety profile and vaccine-induced immune response, avoids interference from pre-existing immunity that occurs with human adenovirus vaccines, and requires only a single administration for induction of immune responses.

Added value of this study

This trial describes the outcomes from the first Sudan Ebola virus-specific vaccine trial evaluating a single dose cAd3-EBO S vaccine, which shows that the vaccine is safe, well tolerated, and immunogenic. We observed a Sudan Ebola virus glycoprotein-specific binding antibody response in 34 (85%) of 40 of participants 4 weeks after vaccination, which was durable in 31 (82%) of 38 of participants up to 48 weeks.

Implications of all the available evidence

The results of this trial support further development of this Sudan Ebola virus-specific vaccine, cAd3-EBO S. The rapid development of a durable antibody response following a single vaccination highlights the suitability of this vaccine in Sudan Ebola virus outbreak responses. Importantly, the cAd3-EBO S vaccine product has been evaluated in more individuals than any other single-dose Sudan Ebola virus-specific vaccine, having completed phase 1 studies in both monovalent and bivalent cAd3-EBO formulations. The results described here, in addition to the previously published cAd3-vectored bivalent EBO vaccine clinical trial results, show that the cAd3-EBO S vaccine is safe and produces durable binding antibody and T-cell responses against the Sudan Ebola virus glycoprotein by 4 weeks after vaccination.

disease.[1] A 2022 outbreak of Sudan Ebola virus in Uganda caused 55 deaths among 142 confirmed infections,[3] a case fatality rate of 34%. Among seven previous Sudan Ebola virus outbreaks in Sudan and Uganda between 1976 and 2012, there were a total of 793 confirmed cases and 426 deaths,[4] resulting in a case fatality rate of 54%.[5] The increased size of outbreaks with consequent spread of filoviruses into additional countries since 2013 emphasises the urgent need to develop vaccines to limit and prevent future filovirus outbreaks.[5] Although two vaccines have attained WHO prequalification status for use against Zaire Ebola virus,[6,7] Zaire Ebola virus-specific vaccines are unlikely to confer cross-protective immune responses against the antigenically distinct Sudan Ebola virus.[8] Therefore, there remains an unmet need for vaccines and therapeutics in Sudan Ebola virus outbreaks.

Currently, the most clinically advanced Sudan Ebola virus-specific vaccine is the chimpanzee adenovirus 3 (cAd3-EBO) vaccine reported here. Preclinically, the cAd3-EBO S vaccine was immunogenic and had protective durability up to 12 months in non-human primates.[9] Multiple phase 1 and 1b trials involving the cAd3 platform in a bivalent Zaire-Sudan cAd3-EBO vaccine have been done (NCT02354404, NCT02231866, NCT02408913, NCT02368119).[10] This bivalent vaccine was shown to have an excellent safety profile and was immunogenic, resulting in both Zaire-specific and Sudan-specific antibodies and T-cell responses by 4 weeks after vaccination.[10] Additional multivalent vaccines are currently undergoing phase 1 clinical testing. The first, a ChAdOx1 biEBOV vaccine, expressing both Zaire and Sudan glycoproteins is being studied in two ongoing phase 1 trials in the UK (NCT05079750) and Tanzania (NCT05301504).[11] The second, a multivalent Ad26.Filo/MVA-BN-Filo vaccine regimen, recently reported results showing safety and immune responses against Zaire, Sudan, and Marburg antigens after completion of the prime-boost regimen (NCT02860650).[12] Additionally, although the extensive clinical testing with the Zaire-specific rVSV-ZEBOV vaccine could be extrapolated for

the Sudan-specific rVSV-SEBOV-GP vaccine, the Sudan Ebola virus-specific product has yet to be evaluated in humans.[13,14] To date, there are no Sudan-specific single-dose vaccines approved for use in endemic areas or during Sudan Ebola virus outbreaks.

The bivalent cAd3-EBO vaccine has promising safety and immunogenicity results in previous trials, however the cAd3-EBO S vaccine has never been evaluated in a monovalent form. The Vaccine Research Center and Walter Reed Army Institute of Research (WRAIR), in partnership with the Makerere University Walter Reed Project (MUWRP), evaluated the monovalent cAd3-EBO S vaccine in Kampala, Uganda. In this trial we evaluated the safety, tolerability, and immunogenicity of cAd3-EBO S in healthy adults following a single dose of either 1×10^{10} particle units (PU) or 1×10^{11} PU of cAd3-EBO S. These doses were found to be safe and immunogenic in previous preclinical and clinical trials evaluating cAd3-vectored vaccines.[10,15,16] Importantly, this trial took place in Kampala, Uganda, and therefore evaluated the vaccine in a population regularly threatened by Sudan Ebola virus outbreaks.

Methods

Study design

This study was a phase 1, open-label, dose-escalation trial examining the safety, tolerability, and immunogenicity of two different doses (1×10^{10} PU and 1×10^{11} PU) of a Sudan Ebola virus chimpanzee adenovirus vector vaccine, cAd3-EBO S, in healthy adults. Scientists at the Vaccine Research Center (National Institute of Allergy and Infectious Disease [NIAID], the US National Institutes of Health) developed the vaccine and sponsored the trial done by MUWRP investigators at the MUWRP Clinical Trial Site in Kampala, Uganda. The study was reviewed and approved by the Makerere University School of Public Health Institutional Review Board.

Participants

Study participants were recruited from the Kampala metropolitan area using Institutional Review Board-approved written and electronic media that explained the trial intervention. Women who were pregnant, breastfeeding, or planning to become pregnant during the first 24 weeks of the trial were excluded from enrolment. Pregnancy was evaluated during screening and at enrolment with a β-human chorionic gonadotropin pregnancy test. Eligible participants were adults aged 18–50 years in good general health by physical examination and laboratory assessments, without previous receipt of an investigational Ebola, Marburg, or cAd3-vectored vaccine. Full inclusion and exclusion criteria are detailed in the trial protocol (appendix pp 34–37). All participants provided written informed consent before study enrollment. An assessment of understanding was completed during consent, where participants had to score at least 90% within three attempts to enrol in the trial.

The description of some parts of the Methods is similar to those of a trial we did for a cAd3-vectored Marburg virus vaccine candidate.[16]

Enrolment and masking

This trial was open label to both participants and clinicians. Two groups were sequentially enrolled according to a dose-escalation plan, each participant receiving a single vaccination on day 0. Study enrolments for the 1×10^{10} PU vaccine dose were limited to one participant per day for the first three participants. Enrolment for the remaining participants in the 1×10^{10} PU dose group occurred following a minimum of 7 days of follow-up and safety review by the protocol safety review team. Enrollment of participants into the 1×10^{11} PU dose group began in the same manner following a minimum of 7 days of follow-up on the last 1×10^{10} PU dose participant and a dose-escalation safety review approval by the protocol safety review team.

Procedures

The recombinant chimpanzee adenovirus 3 Sudan Ebola virus (cAd3-EBO S) vaccine is comprised of a replication-deficient cAd3 vector modified by an E1 region deletion and insertion of an expression cassette for the wild-type glycoprotein sequence from the Sudan Ebola virus Gulu strain. The drug substance was manufactured at Advent (Pomezia, Italy), a subsidiary of Okairos (now GlaxoSmithKline). The drug product (VRC-EBOADC086–00-VP), and diluent (VRC-DILADC065–00-VP) were manufactured according to cGMP regulations at the Vaccine Research Center Pilot Plant, operated by the Vaccine Clinical Materials Program, Leidos Biomedical Research, (Frederick, MD, USA). The investigational product was a sterile, aqueous, buffered solution composed of cAd3-EBO S drug substance filled into single-dose vials at 1×10^{11} PU/mL. This monovalent vaccine was evaluated at two doses in this trial: 1×10^{10} (henceforth 10^{10}) PU, and 1×10^{11} (henceforth 10^{11}) PU. Diluent was added to prepare the 10^{10} PU dose on the day of vaccine administration. The diluent consisted of 10 mmol/L Tris, 10 mmol/L histidine, 5% sucrose (weight per volume), 75 mmol/L sodium chloride, 1 mmol/L magnesium chloride, 0·02% polysorbate 80 (weight per volume), 0·1 mmol/L EDTA, and 0·5% (volume per volume) ethanol.

All vaccinations were given intramuscularly into a deltoid muscle in a 1 mL volume by needle and syringe. Safety monitoring included a 30 min post-vaccination observation period, telephone follow-up the next day, and clinical and laboratory evaluations done at eight follow-up visits over the 48 weeks of the study. Participants self-reported local and systemic symptoms and the use of concomitant medications for 7 days following vaccination. Adverse events were recorded for 28 days following vaccination and were graded according to the US Food and Drug Administration Guidance for

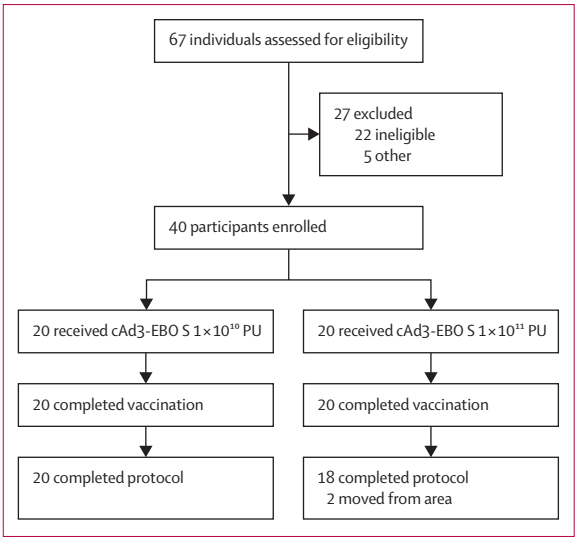

Figure 1: **Trial profile**
Two participants in the 10^{11} PU dose group moved from the area (one after week 4 and one after week 16). Follow-up completed indicates that the participant was followed through the duration of the protocol-specified visits. PU=particle units.

	10^{10} (n=20)	10^{11} (n=20)	Overall (n=40)
Sex			
Male	19 (95%)	11 (55%)	30 (75%)
Female	1 (5%)	9 (45%)	10 (25%)
Age, years			
Mean (SD)	31·5 (6·9)	26·4 (7·3)	29·0 (7·4)
Range	22–44	18–43	18–44
African Ugandan race	20 (100%)	20 (100%)	40 (100%)
BMI, kg/m²*			
Mean (SD)	23·4 (3·5)	23·7 (4·4)	23·6 (3·9)
Range	17·6–31·1	19·0–34·9	17·6–34·9

Data are n (%) unless otherwise specified. Sex and race were self-reported by the participants. Sex was reported based on the sex assigned at birth with the options of male, female, and unknown/not reported. *BMI at enrolment.

Table 1: **Baseline demographics**

Industry,[17] plus additional grading parameters for absolute neutrophil counts and arthralgias (appendix p 127). The relatedness of adverse events to the vaccine product was determined by the investigator, at times informed by discussions with the protocol safety review team.

Outcomes

The primary study endpoints were the safety and tolerability of the cAd3-EBO S vaccine. The safety and tolerability of the cAd3-EBO S vaccine were analysed descriptively in all participants who received the vaccine (ie, modified intent-to-treat analysis), and were defined by the occurrence of solicited local and systemic reactogenicity for the 7 days following vaccination, change from baseline for safety laboratory measures, occurrence of adverse events in the 28 days after vaccination, and the occurrence of serious adverse events and new chronic medical conditions until the last study visit. The secondary endpoints were evaluation of antibody responses to the Sudan glycoprotein insert by ELISA, T-cell responses by intracellular cytokine staining (ICS), and neutralising antibody titres against the cAd3 vector at 4 weeks after vaccination. In addition, an exploratory endpoint assessed the vaccine-induced antibody durability until 48 weeks after vaccination. Peripheral blood mononuclear cells and sera were collected for secondary endpoints at baseline and week 4. Sera were also evaluated for exploratory endpoints at day 3, day 7, week 2, week 8, week 16, week 24, and week 48. The Anti-Sudan GP IgG ELISA, T-cell intracellular cytokine staining, and cAd3 serological assessment assays are described in the appendix (pp 7–8).

Statistical analysis

Statistical analyses were done in a similar form to previous cAd3 studies.[16] Briefly, sample size calculations were based on ability to detect severe adverse events as defined in the protocol with target sample sizes of 20 per group and 40 for the trial overall. Within a group there was a 90% chance to observe at least one serious adverse event if the true rate was no less than 0·109 and a 90% chance of observing no event if the true rate was less than or equal to 0·005.

Positive antibody responses were identified by a finding of significance ($p<0·05$) from a within participant *t* test comparing the triplicate ELISA readings at week 4 to those at baseline for each participant. Group ELISA antibody titres for each dose were expressed as geometric mean titres and compared between timepoints by paired *t* test. An intracellular cytokine staining response was defined as positive if the result of a one-sided Fisher's exact test for the 2×2 table, consisting of positive and negative cells by peptide and negative control, had a $p<0·01$, and the percentage of background subtracted positive cells exceeded the following: as a percentage of CD4 T cells, 0·025% for IFN-α^+, 0·029% for TNF-α^+, and 0·013% for IL-2^+ or as a percentage of CD8 T cells, 0·035% for IFN-α^+, 0·033% for TNF-α^+, and 0·023% for IL-2^+. These baseline data-defined thresholds were determined such that only 1% of the participants had cytokine responses positive for each cytokine (per T-cell subset) at baseline. Comparisons of the percent of participants with positive T-cell responses between groups were done using two-sided Barnard's tests. For each dose, comparisons of background-subtracted non-naive T-cell cytokine responses between week 4 and baseline were done using paired *t* tests.

Comparisons of baseline $\log_{10}$-transformed cAd3 neutralising antibody titres were done by paired *t* test. Comparisons of Sudan Ebola virus glycoprotein-specific antibody titres, non-naive T-cell frequencies, or cAd3 neutralising antibody titres between dose groups were

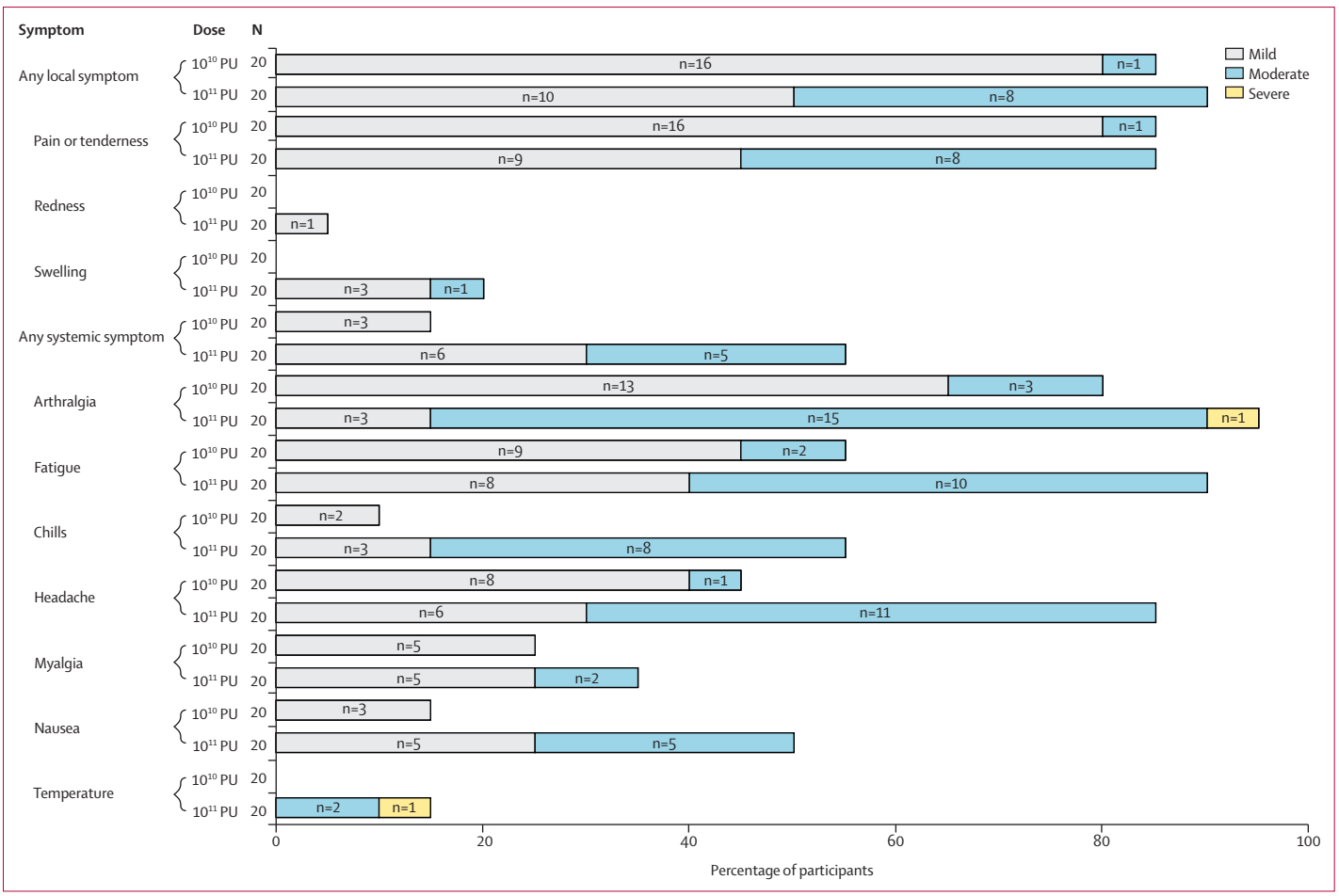

Figure 2: **Maximum local and systemic solicited symptoms in the 7 days following vaccination**
For symptoms persisting more than 1 day, a single count per person at the maximum severity of the symptom was used for the figure.

done per protocol-specified Welch's two-sample *t* test. If datasets were found to have a non-normal distribution, Wilcoxon rank-sum tests were done and reported with the *t* test results. Spearman's correlations were calculated between baseline $\log_{10}$-transformed cAd3 neutralising antibody titres and week 4 Sudan Ebola virus glycoprotein-specific ELISA antibody titres, and week 4 CD4 and CD8 non-naive T-cell responses. Neutralisation assay titres were reported as 90% inhibitory concentration (IC_{90}) and results below the lower limit of detection (ie, ≤12 IC_{90}) were imputed using half the lower limit of detection (ie, 6 IC_{90}). All analyses were done in R version 4.0.4, with DescTools and Barnard packages.

This study is registered at ClinicalTrials.gov, NCT04041570.

Role of the funding source

NIAID funded the study via an interagency agreement with WRAIR and approved the study design. The Vaccine Research Center sponsored and designed the study and did the research assays, data analysis, and data interpretation. The Vaccine Research Center, WRAIR, and MUWRP investigators contributed to the writing of this report.

Results

Between July 22 and Oct 1, 2019, at the Makerere University Walter Reed Project in Kampala, Uganda, 40 healthy adults were enrolled in the study (figure 1). Participants' mean age was 29 years (range 18–44; table 1). Each participant received a single vaccination of cAd3-EBO S at either 10^{10} or 10^{11} PU. All participants completed their secondary endpoint visits, however two (5%) of the 40 participants, both in the 10^{11} PU dose group, moved from the area before completing all follow-up visits. The remaining 38 (95%) participants completed all protocol-specified visits.

The vaccine was safe and generally well tolerated. The most common local symptom was pain or tenderness at the injection site (34 [85%] of 40). Solicited systemic reactogenicity (figure 2) was predominantly mild (16 [40%] of 40) or moderate (18 [45%] of 40). The only exception was one (3%) instance of severe transient fever in a participant the day after receiving cAd3-EBO S at

10^{11} PU. This fever was measured at 103·1°F (39·5°C) and lasted less than 1 day. The most commonly reported systemic symptoms were fatigue (29 [73%] of 40) and headache (26 [65%] of 40). No serious adverse events, onset of new chronic medical conditions, or safety pauses occurred during the trial. Five (13%) participants had an adverse event evaluated as related to vaccination: one (3%) instance of mild dizziness beginning on the day of injection and lasting 2 days, two (5%) instances of leukopenia (one mild and one moderate) beginning 3 days after vaccination (the mild leukopenia was resolved 14 days later and moderate 26 days later), and two (5%) instances of moderate neutropenia beginning on day 3 for one participant (resolved 11 days later) and day 28 for the other (resolved 28 days later; table 2). All adverse events resolved without sequelae.

The cAd3-EBO S vaccine was immunogenic. 34 (85%) of 40 participants developed a positive binding antibody response at 4 weeks after vaccination, the secondary endpoint for the trial (figure 3). The geometric mean titre at 4 weeks (figure 3A; appendix p 5) was significantly (Welch's two-sample *t* test p=0·0012 and Wilcoxon rank sum test p=0·0027) higher after the 10^{11} PU dose (449 EC_{90}, 95% CI 249–809) than after the 10^{10} PU dose (125 EC_{90}, 95% CI 77–201). In exploratory investigations, additional times after vaccination were assayed (figure 3B; appendix p 5). GMT reached near peak levels (277 EC_{90} for 10^{11} PU and 102 EC_{90} for 10^{10} PU) as early as 2 weeks after vaccination (figure 3B), with positive responses in 31 (78%) participants. Additionally, GMTs were found to be significantly greater after 10^{11} PU than 10^{10} PU at most timepoints after vaccination (figure 3B; appendix p 5). The binding antibody responses were durable, with no significant decrease in the GMTs between the week 4 response and the week 48 response. Indeed, 31 (82%) of the 38 participants who completed follow-up maintained their positive responder status up to week 48, the final timepoint evaluated (table 3).

Vaccination with cAd3-EBO S also elicited T-cell responses by 4 weeks after vaccination (figure 4, appendix p 6). 20 (59%, 95% CI 41–75) of 34 participants developed a T-cell response specific for Sudan Ebola virus glycoprotein. Six participants receiving the 10^{11} dose had their T-cell data omitted from the ICS analysis due to failing quality control parameters. 19 (56%, 38–73) of 34 participants were determined to have a Sudan Ebola virus glycoprotein-specific CD4 T-cell response and nine (26%; 13–44) were found to have a Sudan Ebola virus glycoprotein-specific CD8 T-cell response. The responses of all participants with a positive CD4 T-cell response after 10^{11} PU included TNF-α-producing cells ($CD4^+$ TNF^+), significantly (p=0·0078) more than the frequency of $CD4^+$ TNF^+ responders after 10^{10} PU (appendix p 6). Similarly, a greater proportion of 10^{11} participants developed IL-2-producing CD4 T cells after vaccination than 10^{10} recipients (p=0·049). The responses of all participants with a positive CD8 T-cell response included IFN-γ-producing cells ($CD8^+$ IFN^+).

	Vaccination dose	Related adverse events	Severity	Days after vaccination	Days to resolution
1	10^{10}	Leukopaenia	Moderate	3	26
2	10^{10}	Neutropaenia	Moderate	28	29
3	10^{11}	Dizziness	Mild	0	2
4	10^{11}	Leukopaenia	Mild	3	12
5	10^{11}	Neutropaenia	Moderate	3	12

Table 2: **Adverse events related to cAd3-EBO S vaccination, by participant**

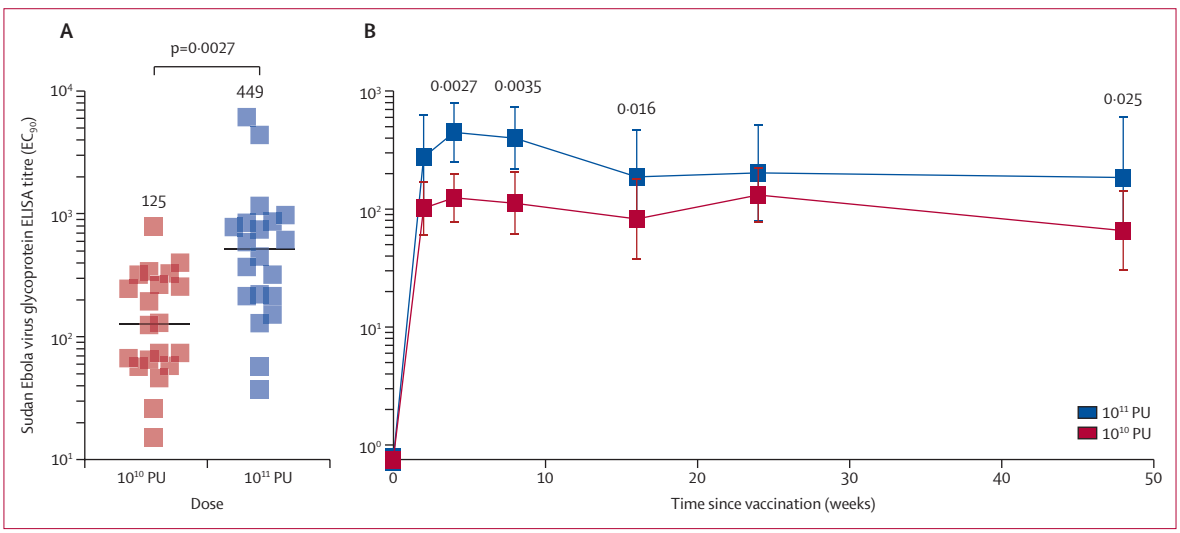

Figure 3: **cAd3-EBO S vaccine elicits increases in antibody titres that are sustained through 48 weeks post-vaccination**
(A) is a detailed view of week 4 (ie, peak) response. In (A), each square indicates a participant's individual titre, and black lines indicate the group GMTs, which are also provided numerically above the population. In (B) squares indicate group GMTs and whiskers are 95% CIs. p values result from a Wilcoxon rank-sum test comparing dose groups. GMT=geometric mean titre.

Among non-naive T cells (figure 4), defined by exclusion of the naive CD45RA+ CCR7+ population, both CD4 and CD8 T-cell populations responsive to Sudan Ebola virus glycoprotein significantly increased after either vaccine dose by the 4-week secondary endpoint. The increase from baseline was significantly greater after the 10^{11} PU dose compared with the 10^{10} PU dose for CD4 T cells (Welch's two-sample *t* test p=0·0052 and Wilcoxon rank sum test p=0·0030), with a similar trend observed for CD8 T cells (Welch's two-sample *t* test p=0·041 and Wilcoxon rank sum test p=0·063). The cAd3-EBO S vaccine elicited T-cell responses in most participants, with patterns of dose dependency and CD4 T-cell predominance as seen for previously published vaccines containing Sudan Ebola virus glycoprotein.[18–20]

To better visualise the vaccine-induced cell-mediated and humoral immune responses, the participants were stratified based on positive responses by ELISA or ICS in a post-hoc analysis (appendix p 2). Three (9%) of 34 participants were non-responders by either assay. 20 (59%) participants had positive responses by more than one parameter, ten (71%) of 14 who received the 10^{11} dose and 10 (50%) of 20 who received the 10^{10} dose. 17 (50%) of the 34 participants had a CD4 T-cell response accompanying their antibody response. Further investigation revealed that week 4 CD4 T-cell responses and ELISA titers were positively correlated (r=0·664 and p<0·0001).

The cAd3-EBO S vaccine elicited cAd3 vector-specific neutralising antibody responses in recipients (appendix p 3). There was a significant (p<0·0001) increase in cAd3 neutralisation titres for all participants from baseline to week 4. However, we found in a post-hoc analysis that the presence of cAd3 vector-specific baseline titres had no effect on week 4 Sudan Ebola virus glycoprotein-specific T cells (CD4 T cells r=–0·269, p=0·12 and CD8 T cells r=–0·25, p=0·16), and were weakly correlated with week 4 Sudan Ebola virus glycoprotein-specific ELISA titres (r=–0·45 and p=0·0041; appendix p 4).

Discussion

In this first report evaluating the monovalent cAd3-EBO S vaccine, we show the safety, tolerability, and immunogenicity of a vaccine that specifically targets Sudan Ebola virus. The results show that cAd3-EBO S was well tolerated and immunogenic at both evaluated doses. Importantly, no serious adverse events were had by the participants in the study. The binding antibody and T-cell responses indicate that the 10^{11} PU dose might offer enhanced immunogenicity, particularly within the first few weeks after vaccination, without a concerning decrease in tolerability. A safe and efficacious Sudan Ebola virus vaccine is urgently needed; especially considering the recent outbreak in Uganda, the fifth such outbreak recorded for the country. These findings support advancing this vaccine to phase 2 and 3 clinical testing.

	Week 4 positive response	Week 48 positive response
10^{10}	17/20 (85%, 62–97)	16/20 (80%, 56–94)
10^{11}	17/20 (85%, 62–97)	15/18 (83%, 59–96)

Data are n/N (%, 95% CI). Positive response defined as a significant increase over baseline titre.

Table 3: **Positive antibody titre response rates via ELISA at weeks 4 and 48**

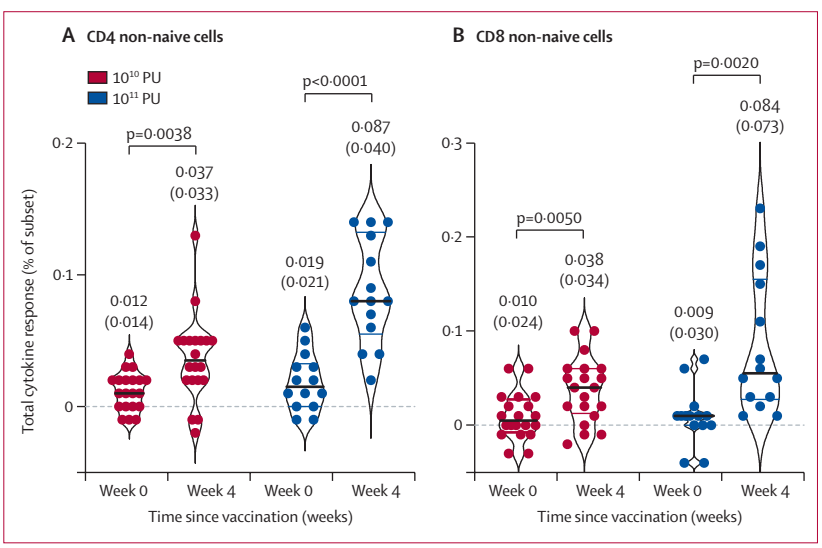

Figure 4: **Frequency of Sudan Ebola virus glycoprotein-specific non-naive CD4 and CD8 T cells increases by 4 weeks after vaccination**

Percent of background-subtracted non-naive T cells producing any one of the three tested cytokines (IFN-γ, IL-2, or TNF-α) in response to Sudan Ebola virus glycoprotein peptide stimulation at baseline and 4 weeks post-vaccination by dose group. (A) shows CD4 T cells. (B) shows CD8 T cells. Above each violin plot, the group mean (SD) is indicated. n=20 for 10^{10} and 14 for 10^{11} at each time point. P values result from paired *t* tests comparing background-subtracted non-naive T-cell frequencies from baseline to week 4.

The results reported here represent the first monovalent Sudan vaccine clinical trial published to date. Previously, a bivalent cAd3 vaccine (cAd3-EBO) used the glycoproteins from both Sudan Ebola virus and Zaire Ebola virus.[10] The safety and immunogenicity findings of this monovalent vaccine trial are consistent with the findings of the previous bivalent vaccine trial.[10] Although the assays used between the trials were not identical and cannot be directly compared, similar dose-dependent trends in antibody responses were observed in both trials at week 4, including a significant difference between dose groups (p=0·01 in the bivalent trial). Furthermore, a similar frequency of participants had a positive response to the Sudan Ebola virus glycoprotein after the monovalent (85%) and bivalent vaccines (70–80%).[10]

In additional clinical trials, Zaire Ebola virus and Sudan Ebola virus glycoproteins were tested in DNA or Ad5 vectored platforms.[18–20] These bivalent vaccines had similar safety and tolerability to cAd3-EBO S but had suboptimal immunogenicity, which meant that multiple injections were required for the DNA vaccine platforms.[19,20] In the Ad5-vectored vaccine trial, individuals with preexisting Ad5 antibody titres had significantly lower

vaccine-induced responses.[18] Consequently, the cAd3 vector was chosen due to low cAd3 seropositivity worldwide.[21,22] Nevertheless, the effect of pre-existing cAd3 titres on week 4 immunogenicity after cAd3-EBO S was assessed. A weak negative correlation between baseline cAd3 neutralising antibody titres and week 4 Sudan Ebola virus glycoprotein-binding antibody titres was the only significant effect on vaccine-induced responses observed in this trial. Similar vaccine-induced cAd3 neutralisation antibody profiles and weak correlations have been reported previously for cAd3-vectored vaccines.[10,16] Together the data suggest that receipt of the cAd3-EBO S vaccine will not preclude immunogenicity of subsequent cAd3-vectored vaccination.

Ebola components have also been evaluated in trials using MVA-BN-Filo (which contains Zaire, Sudan, and Marburg antigens) as a boost after cAd3 or Ad26 vector-based vaccines.[23,24] Strong humoral responses were noted in those trials, indicating that recombinant adenovirus-based vaccines can be successfully used in heterologous prime-boost regimens.[12] However, the 56-day interval in the prime-boost regimen might not be ideal for outbreak settings. Although strong Sudan Ebola virus-specific responses have been observed following MVA-BN-Filo boost,[12] differences in immune assays and readouts confound direct comparisons between the trials' results. The strong immunogenicity and durability of the single-dose cAd3-EBO S vaccine in this trial indicate that the vaccine could be an optimal monovalent vaccine to use in outbreak responses, amenable to potential boosting during non-outbreak periods.

Correlates of protection against Sudan Ebola virus in humans remain to be established.[25] Studies investigating the correlates of protection for the cAd3-EBO S vaccine in non-human primates are currently ongoing. However, non-human primate studies involving cAd3-vectored vaccines for Zaire Ebola virus and Marburg have indicated that greater binding antibody titres are associated with increased survival.[15,26] The cAd3-EBO S vaccine in this trial elicited rapid and robust binding antibody responses in 85% of participants at week 4, which remained durable in 82% of participants up to 48 weeks. These antibody responses were near peak levels just 2 weeks after vaccination, indicating a rapid onset of immunity, which is crucial in outbreak-response scenarios. Notably, binding antibody responses were also durable to 48 weeks, which is concordant with previous reports that viral-vectored vaccines induce robust, long-lived immunity.[27] Persisting immune responses might facilitate use of a vaccine not only for protection of those who have close contact with people who are infected during outbreaks, but also for vaccination in endemic areas, particularly for those with potential episodic occupational risk such as health-care workers. Future trials of this vaccine should aim to explore this important finding.

Although the role of T-cell responses in animal models of Sudan Ebola virus vaccination and challenge studies have yet to be reported, studies of Sudan Ebola virus-infected human samples suggest that CD8 T cells are important in survival after Sudan Ebola virus infection and CD4 T cells are important in neutralising antibody responses.[28–30] An early T-cell recall response to an Ebola infection in vaccinated individuals would be expected to decrease peak viral load, accelerate development of antibody titres, and lessen the pathological effect of infection.[31] In this trial, half of participants responded to the vaccine with both humoral and CD4 T-cell responses. Furthermore, the week 4 glycoprotein-specific ELISA titres and frequency of non-naive CD4 T cells were correlated. These data support a role for CD4 T cell help in the development of humoral immune responses to the cAd3-EBO S vaccine. However, controlled studies in animal models are needed to further define the potential role of T cells in Sudan Ebola virus infection.

There are several limitations to this trial. This phase 1 study was designed and powered to evaluate the safety and tolerability of the vaccine candidate and not to evaluate the efficacy of cAd3-EBO S. The small trial size and absence of placebo control group limit the power of our statistical statements and conclusions on vaccine-induced immune response and optimal dose. In addition, this trial occurred at a single trial site, potentially limiting the generalisability of the results. The trial enrolment was biased towards male participants, which confounded our efforts to perform a sex-based analysis of the immunogenicity. Biases in safety assessments also cannot be ruled out, especially due to the open-label study design. The open-label design allowed trial participants and site staff to be aware of vaccine and dose designation, which could have impacted self-reported reactogenicity. In addition, these are the first Sudan Ebola virus-specific trial results to be published, meaning that only limited reference can be made with existing literature and conclusions are difficult to draw based on any such comparisons.

Ebola disease outbreaks are sporadic, unpredictable, and accelerating in frequency with consequent substantial threat to global health, yielding a growing imperative to develop effective tools to prevent and treat disease caused by multiple *Filoviridae* viruses. A potent, tolerable, single dose vaccine such as cAd3-EBO S that offers both acute and durable immune responses might be capable of both saving individual lives and reducing viral transmission rates, underscoring the urgent need to expedite advanced testing of this vaccine. The cAd3-EBO S monovalent vaccine has been evaluated in this phase 1 trial and a completed Sabin Vaccine Institute-sponsored phase 1b trial (NCT04723602). A Sabin Vaccine Institute-sponsored phase 2 trial in Uganda and Kenya is being developed, with enrolment planned for later in 2023. The data we present in this manuscript support the utility of the cAd3 vaccine platform in Sudan Ebola virus outbreak responses.

Contributors

BM is the primary investigator of the RV 508 clinical trial reported in this manuscript. JGG, ABM, RAK, JRM, NJS, HK, JAA, and JEL contributed to trial conception and design. BM, KVH, ARH, AMO-V, MO'C, BCL, EEC, PAS, and AP contributed to manuscript drafting. BM, GVY, CA, MO'C, SS, LAE, ELS, PTS, MFA, IJG, LAH, JGS, PJMC, FHM, PM, SHP, SPH, SV, AP, NJS, and HK contributed to the protocol and regulatory support. BM, PN, FK, IN, AW, AT, EM, JN, MRG, IJG, LAH, TM, JC, CNMD, MB, MP, PAS, BCL, AP, HK, and JAA contributed to the investigation and sample collection. BM, GVY, CA, MO'C, KM, MRG, IJG, LAH, AP, HK, and JAA contributed to data collection and review. KVH, ARH, AMO-V, MO'C, MH, TM, RH, SRG, EEC, AP, NJS, and JAA contributed to data analysis and interpretation. KVH, ARH, AMO-V and LS contributed to figure design. All authors contributed to manuscript revision and final approval. BM, KVH, ARH accessed and verified the data in this manuscript. All authors were responsible for the decision to submit for publication.

Declaration of interests

NJS is listed on patents involving cAd3-vectored vaccines. All other authors declare no competing interests.

Data sharing

Data generated in this study is available as de-identified participant data on ClinicalTrials.gov (NCT04041570). The study protocol, statistical analysis plan, and informed consent form are available on ClinicalTrials.gov. Additional data might be made available upon reasonable request to the corresponding author.

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References

1 Centers for Disease Control and Prevention. Ebola disease distribution map: cases of ebola disease in Africa since 1976. 2023. https://www.cdc.gov/vhf/ebola/history/distribution-map.html (accessed April 21, 2023).
2 Takada A. Ebolavirus and other filoviruses. In: Rezaei N, ed. Encyclopedia of infection and immunity. Amsterdam: Elsevier; 2022: 292–300.
3 WHO. Disease outbreak news; Ebola disease caused by Sudan Ebola virus—Uganda. 2023. https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON433 (accessed July 5, 2023).
4 Kuhn JH, Amarasinghe GK, Perry DL. Filoviridae. In: Howley PMK, David M, eds. Emerging viruses, 7th edn. Philadelphia, PA: Wolters Kluwer; 2021: 449–503.
5 Jacob ST, Crozier I, Fischer WA 2nd, et al. Ebola virus disease. *Nat Rev Dis Primers* 2020; **6:** 13.
6 WHO. ERVEBO. 2019. https://extranet.who.int/pqweb/content/ervebo (accessed April 4, 2023).
7 WHO. Zabdeno. 2021. https://extranet.who.int/pqweb/content/zabdeno (accessed April 4, 2023).
8 Jones SM, Feldmann H, Ströher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med* 2005; **11:** 786–90.
9 Finian A. Sabin ChAd3 Sudan vaccine. 2023. https://cdn.who.int/media/docs/default-source/blue-print/sudan-ebolavirus-vaccines-summary-deliberations_jan-2023-final.pdf?sfvrsn=26f4d5f4_7 (accessed July 6, 2023).
10 Ledgerwood JE, DeZure AD, Stanley DA, et al. Chimpanzee adenovirus vector Ebola vaccine. *N Engl J Med* 2017; **376:** 928–38.
11 University of Oxford. Ebola vaccine to begin human trials. 2021. https://www.ox.ac.uk/news/2021-11-11-ebola-vaccine-begin-human-trials (accessed Oct 25, 2022).
12 Bockstal V, Shukarev G, McLean C, et al. First-in-human study to evaluate safety, tolerability, and immunogenicity of heterologous regimens using the multivalent filovirus vaccines Ad26.Filo and MVA-BN-Filo administered in different sequences and schedules: a randomized, controlled study. *PLoS One* 2022; **17:** e0274906.
13 Bache BE, Grobusch MP, Agnandji ST. Safety, immunogenicity and risk-benefit analysis of rVSV-DeltaG-ZZaire Ebola virus-glycoprotein (V920) Ebola vaccine in phase I–III clinical trials across regions. *Future Microbiol* 2020; **15:** 85–106.
14 Coller B-AG, Lapps W, Yunus M, et al. Lessons Learned from the Development and Roll-Out of the rVSVΔG-ZEBOV-GP *Zaire ebolavirus* Vaccine to Inform Marburg Virus and *Sudan ebolavirus* Vaccines. *Vaccines (Basel)* 2022; **10:** 1446.
15 Stanley DA, Honko AN, Asiedu C, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nat Med* 2014; **20:** 1126–29.
16 Hamer MJ, Houser KV, Hofstetter AR, et al. Safety, tolerability, and immunogenicity of the chimpanzee adenovirus type 3-vectored Marburg virus (cAd3-Marburg) vaccine in healthy adults in the USA: a first-in-human, phase 1, open-label, dose-escalation trial. *Lancet* 2023; **401:** 294–302.
17 US FDA. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. 2007. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical (accessed July 5, 2023).
18 Ledgerwood JE, Costner P, Desai N, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. *Vaccine* 2010; **29:** 304–13.
19 Sarwar UN, Costner P, Enama ME, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. *J Infect Dis* 2015; **211:** 549–57.
20 Kibuuka H, Berkowitz NM, Millard M, et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial. *Lancet* 2015; **385:** 1545–54.
21 Colloca S, Barnes E, Folgori A, et al. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. *Sci Transl Med* 2012; **4:** 115ra2.
22 Quinn KM, Da Costa A, Yamamoto A, et al. Comparative analysis of the magnitude, quality, phenotype, and protective capacity of simian immunodeficiency virus gag-specific CD8+ T cells following human-, simian-, and chimpanzee-derived recombinant adenoviral vector immunization. *J Immunol* 2013; **190:** 2720–35.
23 Tapia MD, Sow SO, Lyke KE, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis* 2016; **16:** 31–42.
24 Agnandji ST, Loembe MM. Ebola vaccines for mass immunisation in affected regions. *Lancet Infect Dis* 2022; **22:** 8–10.
25 Rudge TL Jr, Machesky NJ, Sankovich KA, et al. Assays for the evaluation of the immune response to Marburg and Ebola Sudan vaccination-Filovirus animal nonclinical group anti-Marburg virus glycoprotein immunoglobulin g enzyme-linked immunosorbent assay and a pseudovirion neutralization assay. *Vaccines (Basel)* 2022; **10:** 1211.

26 Hunegnaw R, Honko AN, Wang L, et al. A single-shot ChAd3-MARV vaccine confers rapid and durable protection against Marburg virus in nonhuman primates. *Sci Transl Med* 2022; **14:** eabq6364.

27 Woolsey C, Geisbert TW. Current state of Ebola virus vaccines: a snapshot. *PLoS Pathog* 2021; **17:** e1010078.

28 Sanchez A, Lukwiya M, Bausch D, et al. Analysis of human peripheral blood samples from fatal and nonfatal cases of Ebola (Sudan) hemorrhagic fever: cellular responses, virus load, and nitric oxide levels. *J Virol* 2004; **78:** 10370–77.

29 Sanchez A, Wagoner KE, Rollin PE. Sequence-based human leukocyte antigen-B typing of patients infected with Ebola virus in Uganda in 2000: identification of alleles associated with fatal and nonfatal disease outcomes. *J Infect Dis* 2007; **196** (suppl 2)**:** S329–36.

30 Sobarzo A, Stonier SW, Herbert AS, et al. Correspondence of neutralizing humoral immunity and CD4 T Cell responses in long recovered Sudan virus survivors. *Viruses* 2016; **8:** 133.

31 Perdomo-Celis F, Salvato MS, Medina-Moreno S, Zapata JC. T-cell response to viral hemorrhagic fevers. *Vaccines (Basel)* 2019; **7:** 11.